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Transfection of DNA in inner ear cells using functionalized calcium phosphate nanoparticles

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BACKGROUND / AIMS

Cochlear implants (CI) function by electrical stimulation of the spiral ganglion neurons (SGN) and are well known devices for regaining the ability to hear. Among other things, the efficiency of CI depends on the duration of deafness and the severity of the degeneration of SGN.

To enhance the maintenance and regeneration of SGN, this project aims to develop a chemobiological coating of CI-electrodes. For this purpose biocompatible calcium phosphate nanoparticles (CaP) will be functionalized with DNA encoding neurotrophic factors.

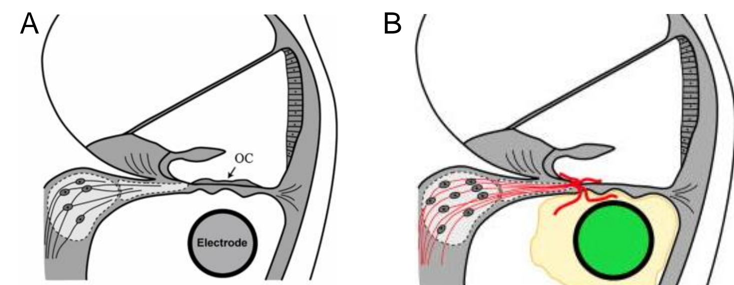


Fig. 1: Schematic structure of the cochlea. (A) shows an exemplary implanted CI-electrode in the scala tympani. Our project aims to develop a coating to maintain and regenerate the SGN (B). In theory this should improve the innervation of the SGN by the electrode.

Methods

The CaP were prepared by a precipitation method via mixing calcium nitrate and diammonium hydrogen phosphate and are functionalized by protein-encoding plasmid DNA (pDNA). Furthermore, the SGN of new born rats were dissected and used as model for uptake and transfection experiments.

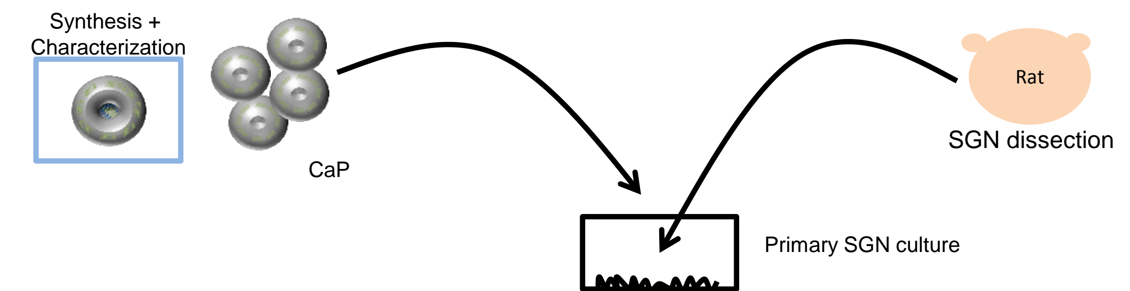


Fig. 2: The synthesis and characterization of CaP was performed in the Institute of Inorganic Chemistry at the University of Duisburg-Essen. Then SGN of new born rats were dissected, cultivated and treated with CaP up to five days.

Results

Synthesis and characterization of triple shell calcium phosphate nanoparticles (CaP)

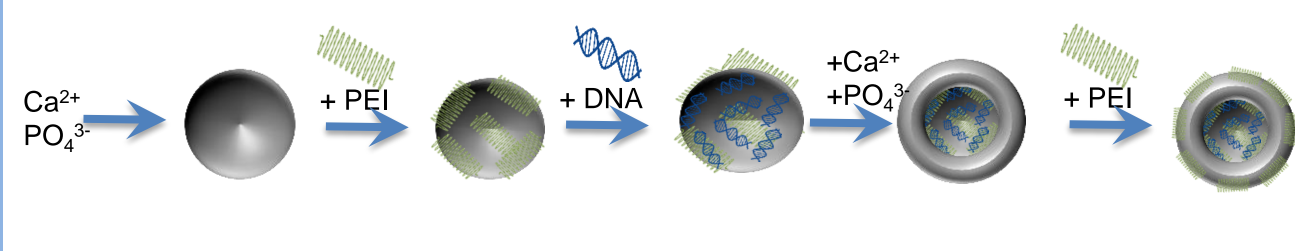
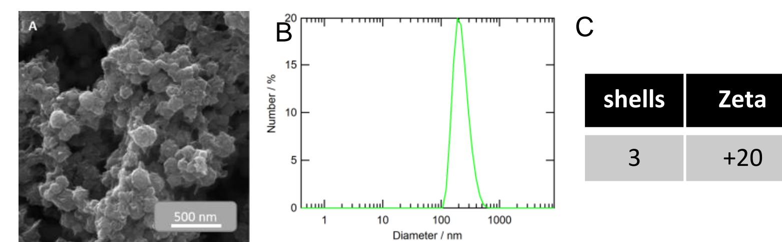


Fig. 3: CaP nanoparticles were precipitated by dropwise addition of Ca^{2+} and PO_4^{3-} solutions. A defined volume was than combined with pDNA solution, stabilizing the particles. For cell studies with dispersions, triple-shell particles were precipitated with PEI and DNA, additional protection of the DNA was accomplished by a layer of CaP and polyethylenimine (PEI) on the surface.



shells	Zeta
3	+20

Fig. 4: Characterization of CaP by SEM (A), dynamic light scattering (B) and measurement of the zeta-potential (C). All CaP used had a size of 100nm and a positive zeta-potential to provide an facilitate the uptake of CaP by cells.

Dissection of rat spiral ganglion neurons

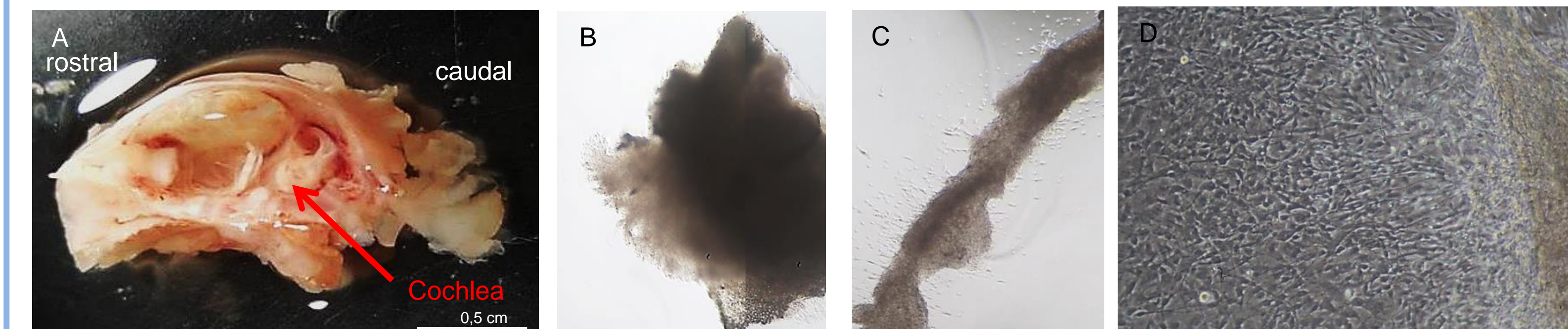


Fig. 5: Microdissection of SGN explants of rat p3-7: At p3-7 rat pups were sacrificed. The head was dissected by a sagittal cut, the brain was removed so that the cochlea was visible (A). Carefully the cochlea was separated from the scalp and the bone surrounding the cochlea as well as the scala tympani and vestibule were removed (result see B). The scala media was enrolled and separated from the modiolus. This explant was cultured on a poly-D-lysin with laminin coating (C). After four days of culture (D) an outgrowth of neuronal cells as well as supporting cells took place. For further analysis pictures of this cell layer were taken.

Spiral ganglion neurons and non-neuronal cells are taking up CaP-FITC

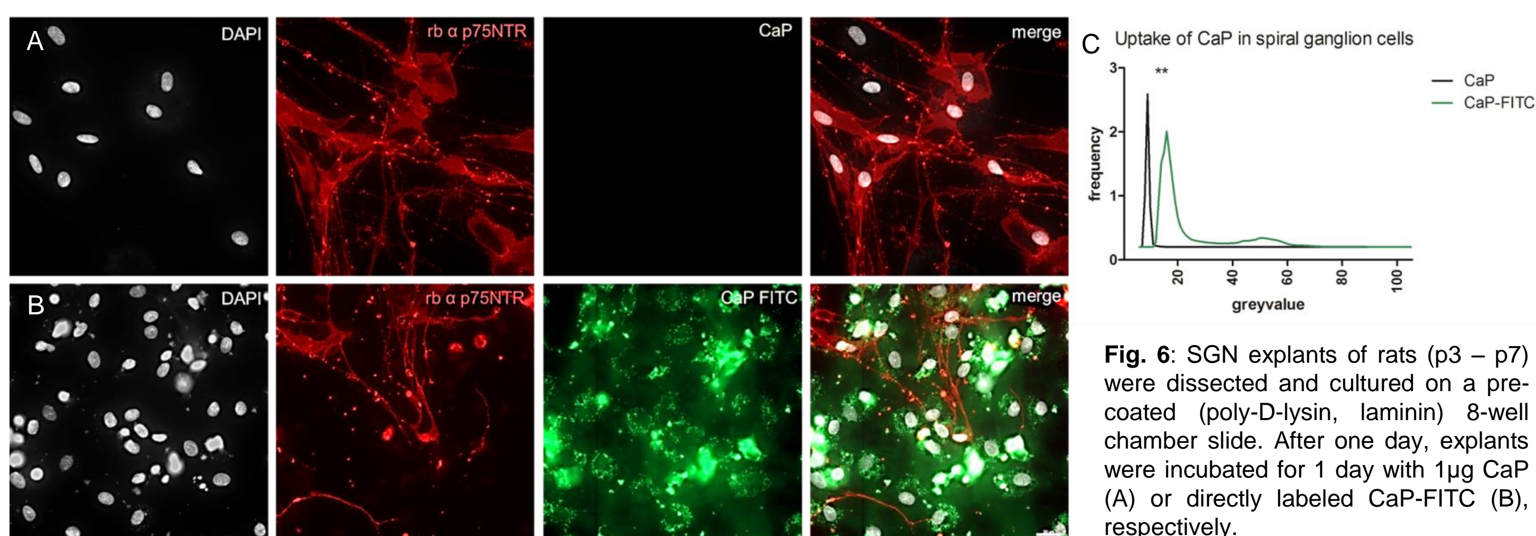


Fig. 6: SGN explants of rats (p3 – p7) were dissected and cultured on a pre-coated (poly-D-lysin, laminin) 8-well chamber slide. After one day, explants were incubated for 1 day with 1µg CaP (A) or directly labeled CaP-FITC (B), respectively. Afterwards cells were fixed and stained for p75NTR, a neurotrophin receptor that was used to identify Schwann-cells. Images were taken with a 63x objective. The Scale bar = 20µm. (C) The histogram shows the frequency of each grey value (FITC) after CaP and CaP-FITC treated SGN explants, which were analyzed with Image J.

Spiral ganglion neurons and non-neuronal cells can be transfected with CaP-eGFP DNA

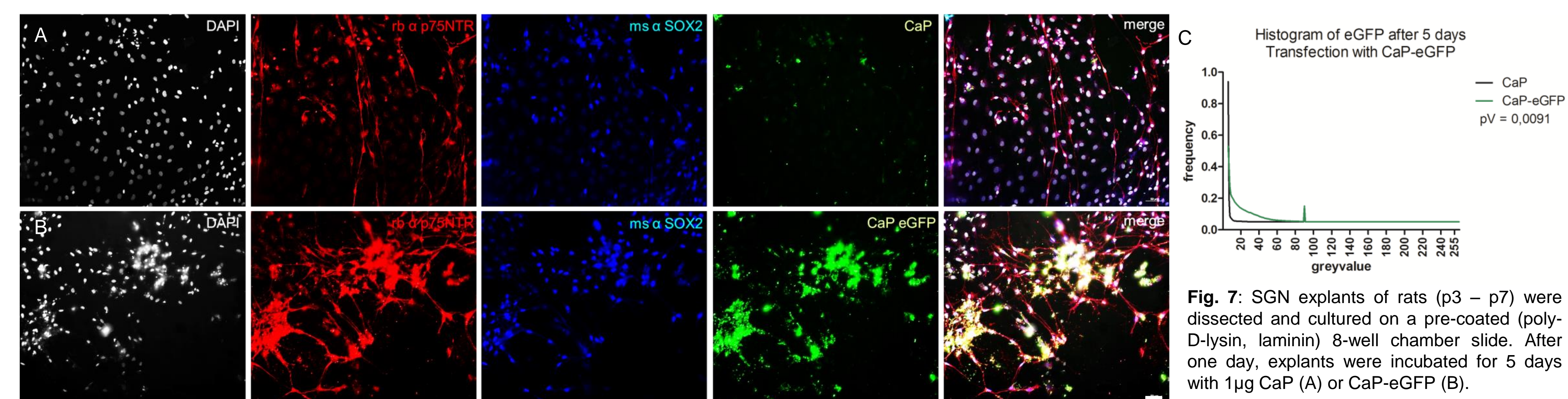


Fig. 7: SGN explants of rats (p3 – p7) were dissected and cultured on a pre-coated (poly-D-lysin, laminin) 8-well chamber slide. After one day, explants were incubated for 5 days with 1µg CaP (A) or CaP-eGFP (B). After every second day medium was changed. (A) Afterwards cells were fixed and stained for p75NTR, a neurotrophin receptor that was used to identify Schwann-cells. Images were taken with a 40x objective. Scale bar = 50µm. (C) The histogram shows the frequency of each grey value (GFP) after CaP and CaP-eGFP treated SGN explants, which were analyzed with Image J.

Summary

Functionalized calcium phosphate nanoparticles are able to transfect a primary rat spiral ganglion neuron culture.

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